

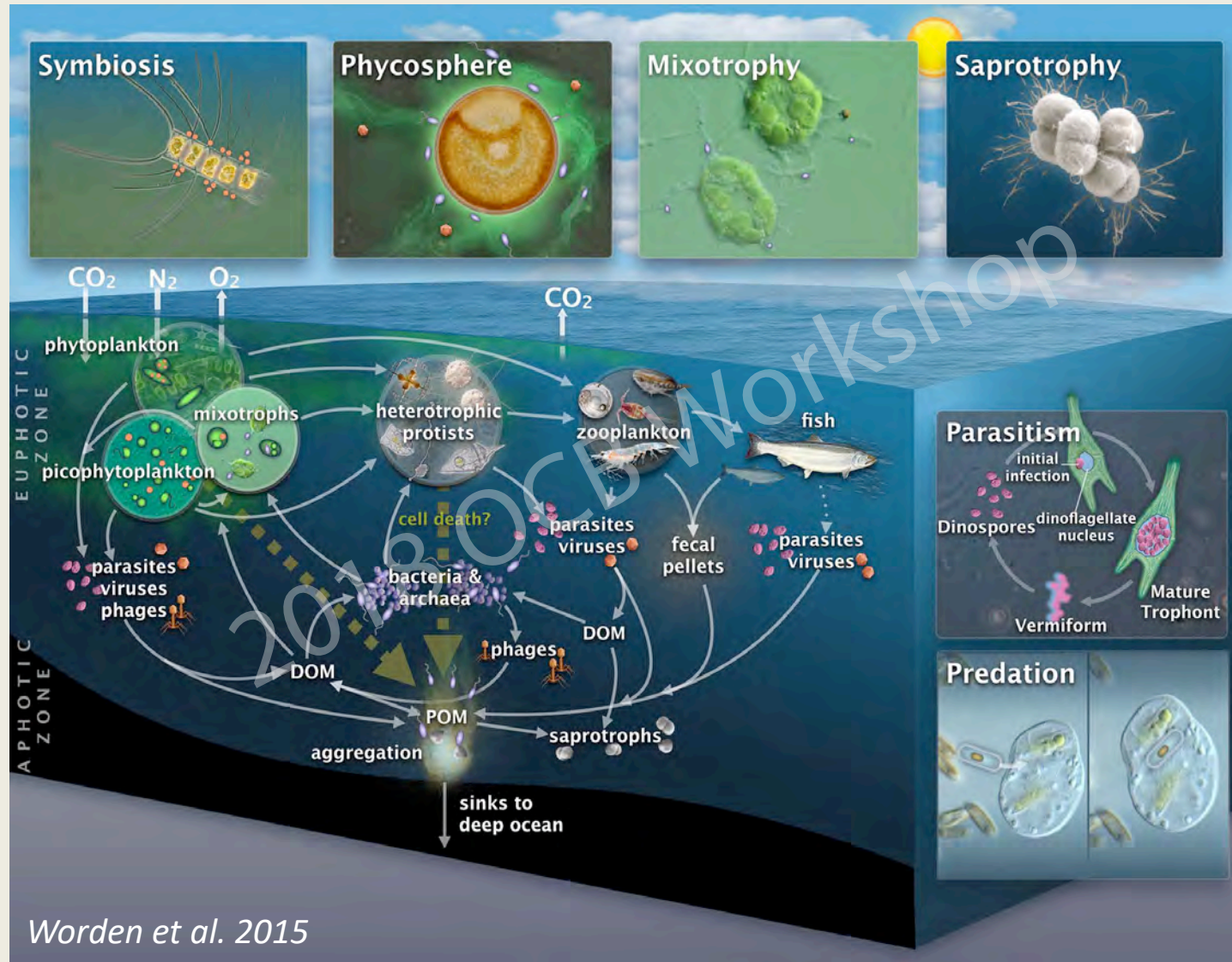
Emerging Roles of Protists in Deep Ocean and O₂-depleted Marine Habitats and Implications for Carbon and other Nutrient Cycling

V. Edgcomb

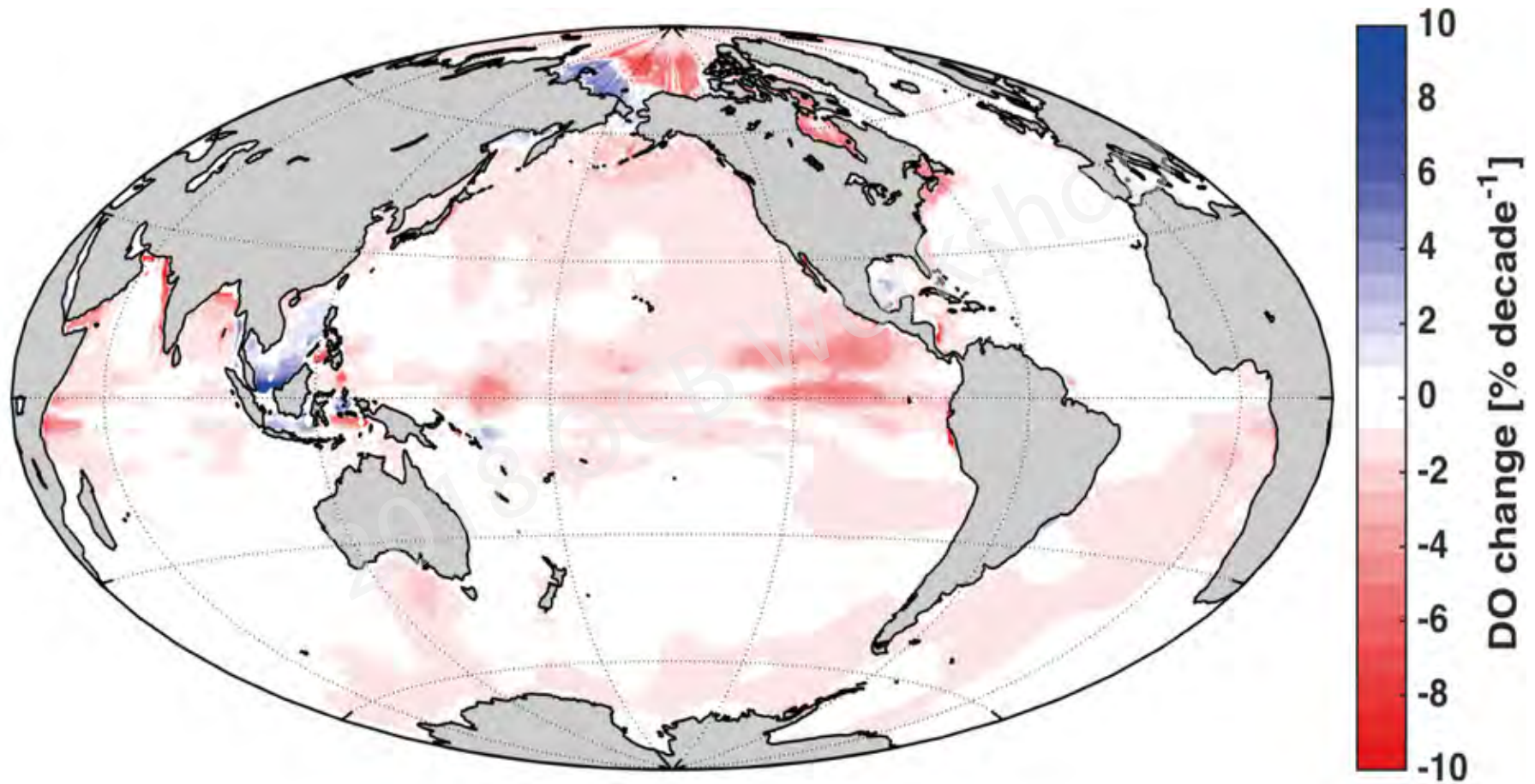
Woods Hole Oceanographic Institution



Protist interactions play crucial roles in the flow of carbon in the ocean



Global decline in dissolved O₂ over past 5 decades



Schmidtko et al. 2017 *Nature*

Protist-prokaryote associations are common in anoxic and O₂-poor marine and FW habitats



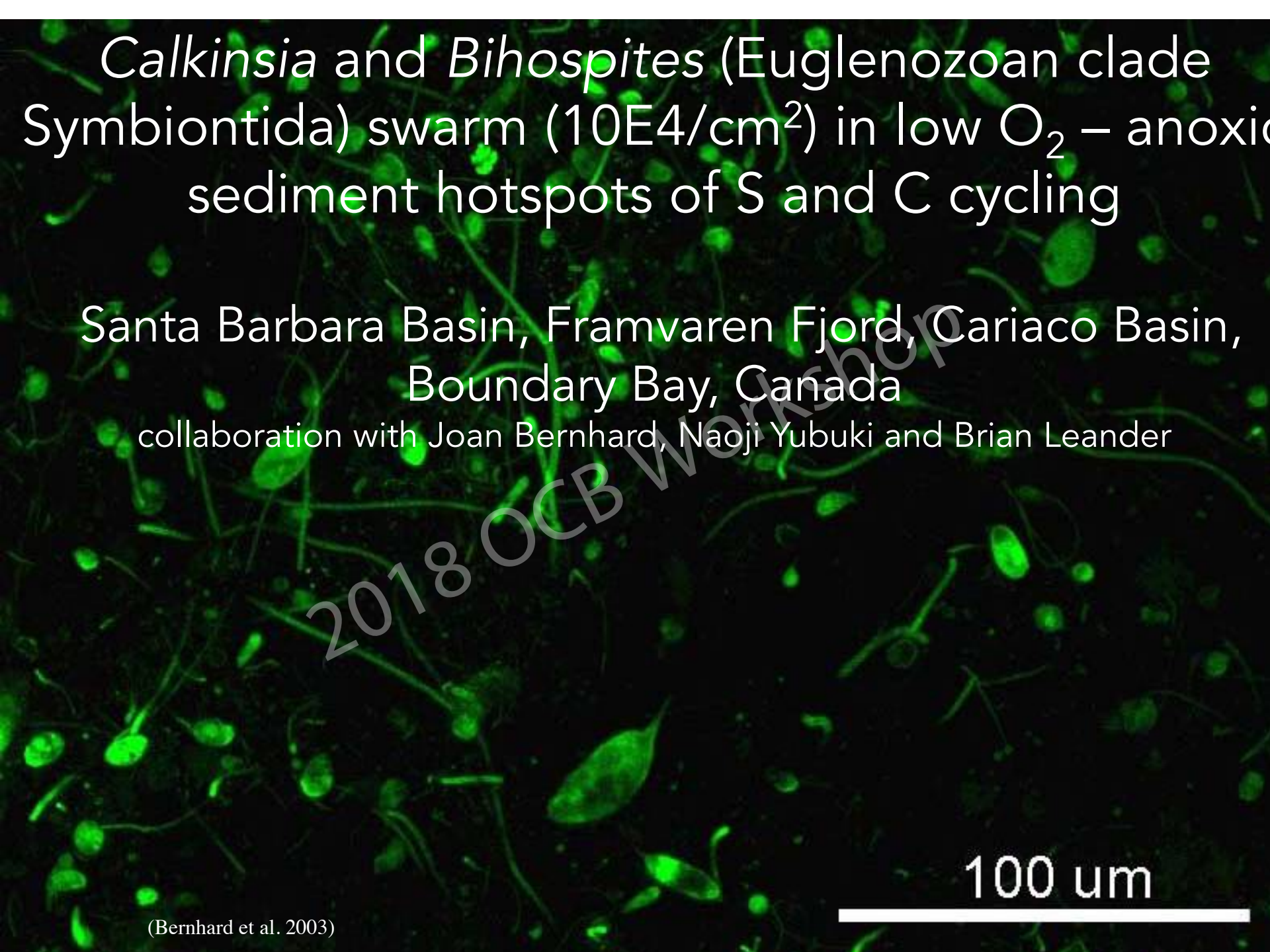
e.g., Van Bruggen et al. 1983; Jones et al. 1987; Fenchel and Finlay 1991; Embley et al. 1992; Embley and Finlay 1993; 1994; Fenchel and Bernard 1993; Nowack and Melkonian 2010; Edgcomb et al., 2011; Orsi et al., 2012; Bernhard et al., 2000

Calkinsia and *Bihospites* (Euglenozoan clade Symbiontida) swarm ($10E4/cm^2$) in low O_2 – anoxic sediment hotspots of S and C cycling

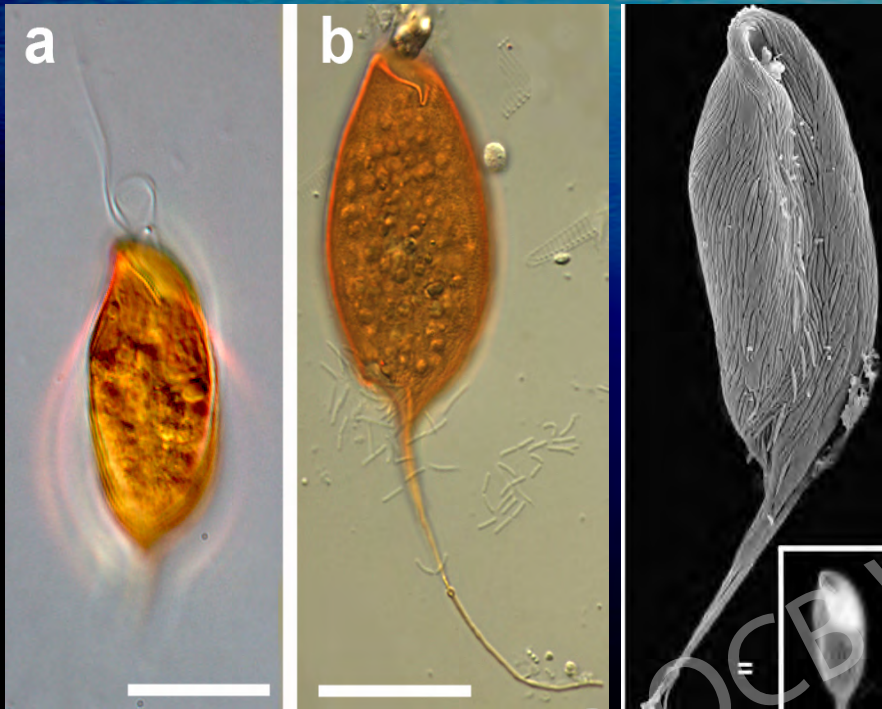
Santa Barbara Basin, Framvaren Fjord, Cariaco Basin,
Boundary Bay, Canada

collaboration with Joan Bernhard, Naoji Yubuki and Brian Leander

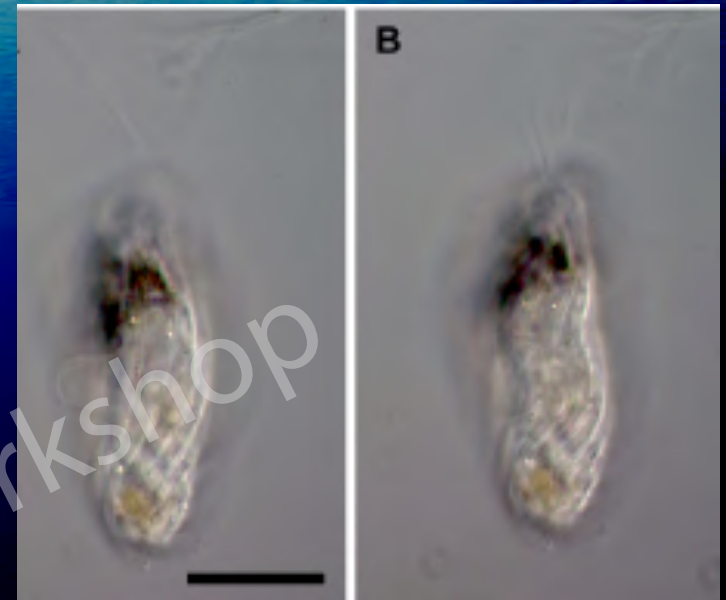
100 μm



Calkinsia SBB

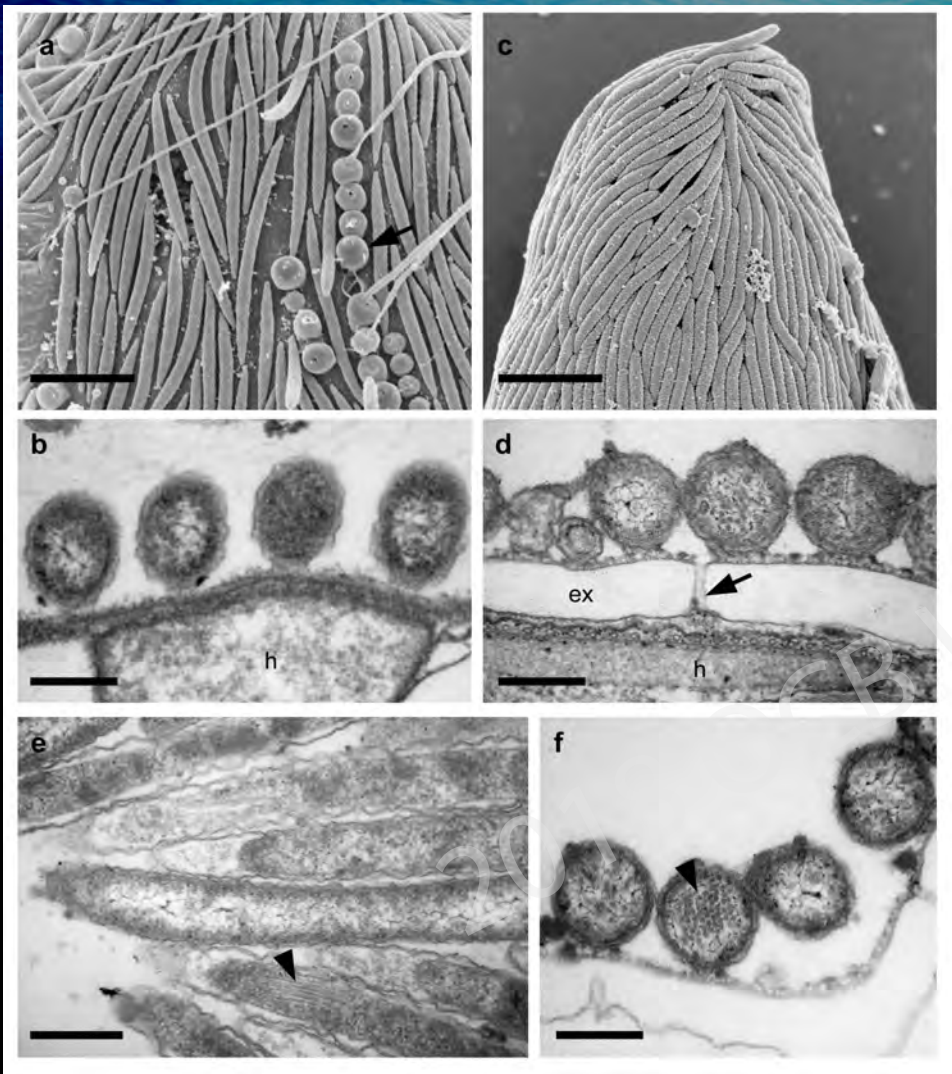


Bihospites Boundary Bay



- Autotrophic, nitrate-reducing, sulfide oxidizing epibionts related to *Arcobacter*
- Epibionts may utilize H_2 in addition to sulfide as e- donor, enhancing function of host hydrogenosomes
- Host may locate ectobionts within biogeochemical gradients to maximize growth

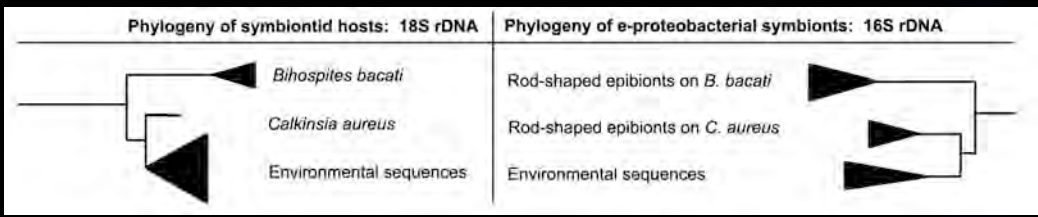
Bihospites (Boundary Bay) *Calkinsia* (SBB)



Epibionts connected to plasma membrane by glycocalyx with conduiting through extracellular matrix

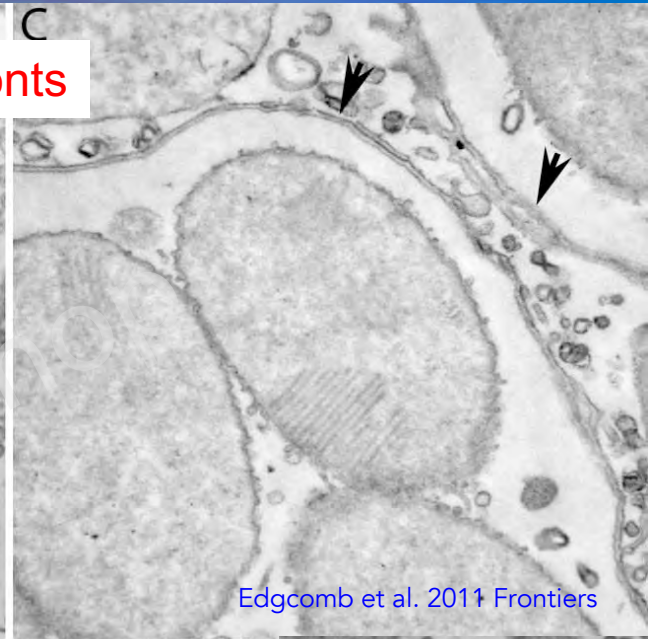
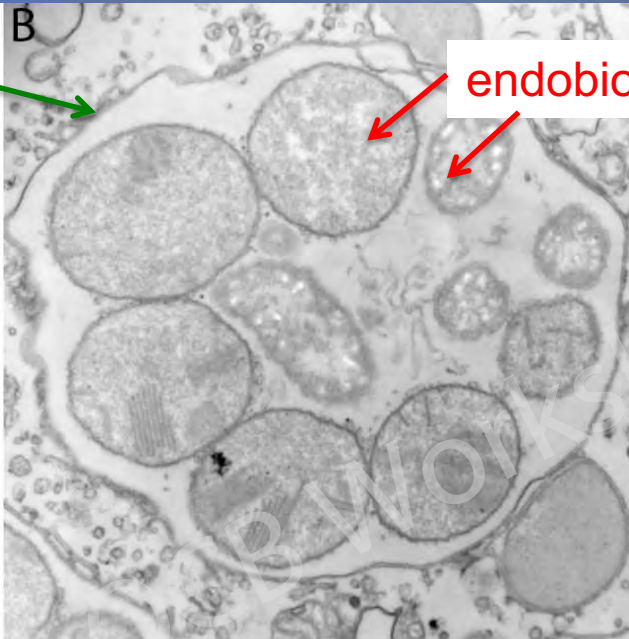
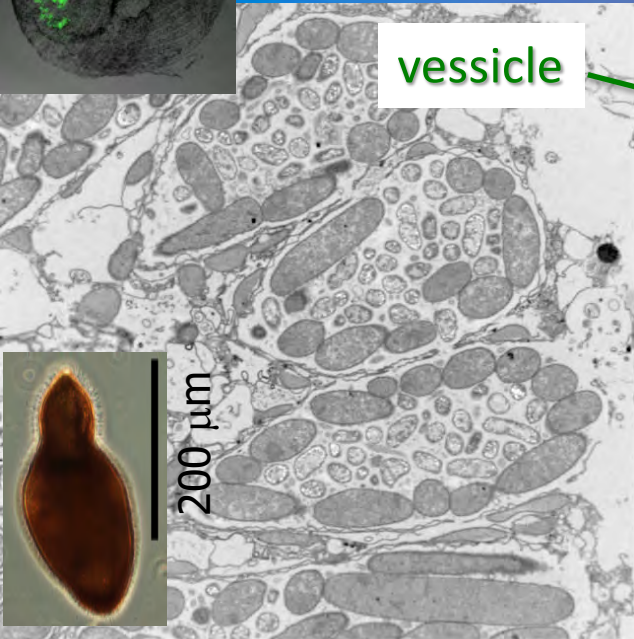
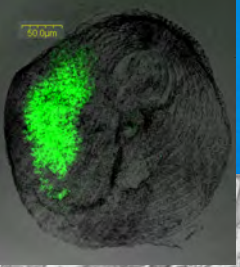
Hydrogenosome-like organelles under plasma membrane

Co-evolutionary history between the 2 sets of closely related partners?

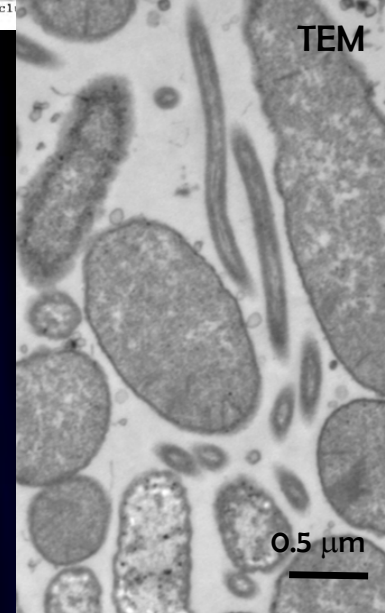


congruent tree topologies

Santa Barbara ciliate with multiple internal bacterial and archaeal endobionts

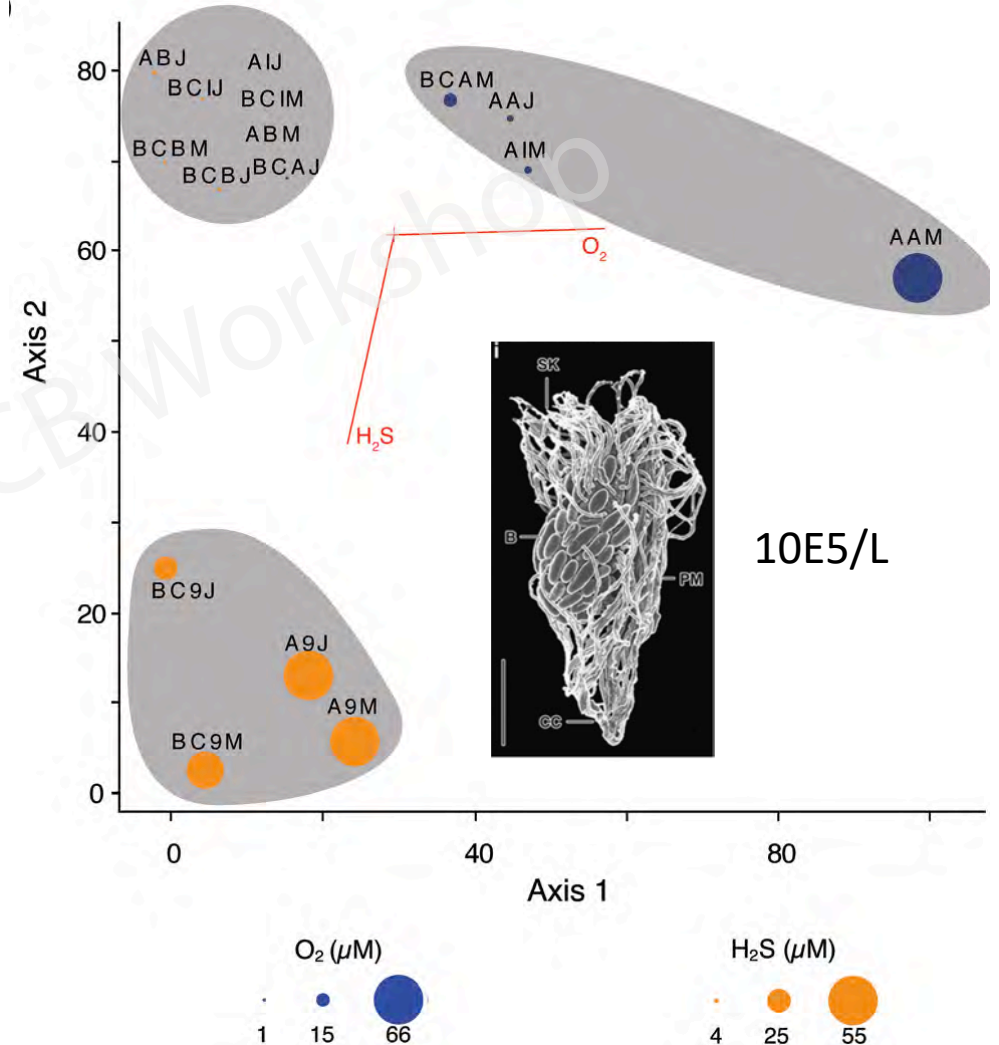
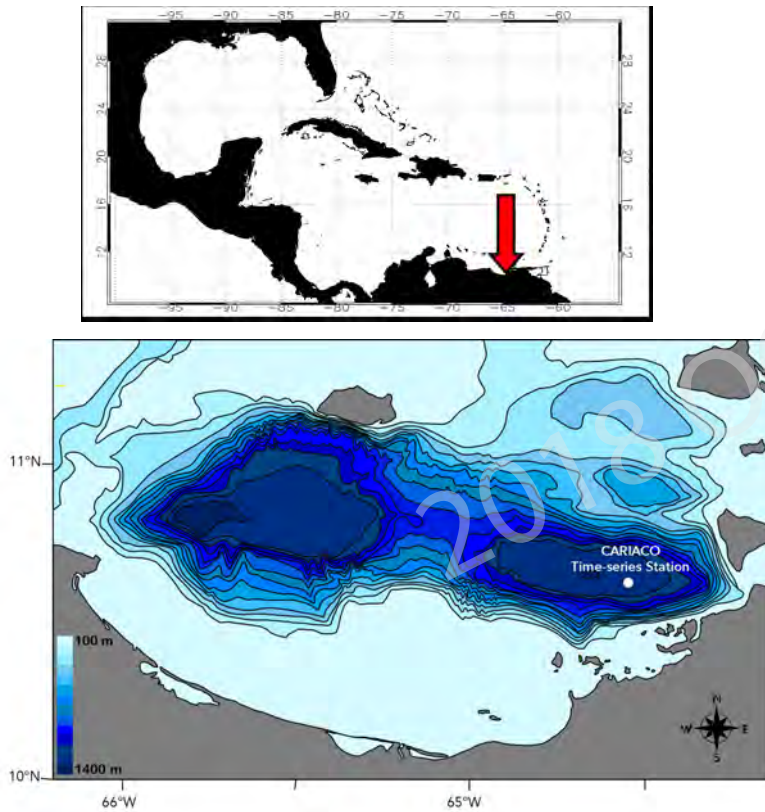


4000.tif Cal: 0.015 micron/pix 2 microns 20k whole.tif Cal: 0.003 micron/pix 500 nm stacked membrane and membrane of cilia Print Mag: 88300x @ 7.0 in

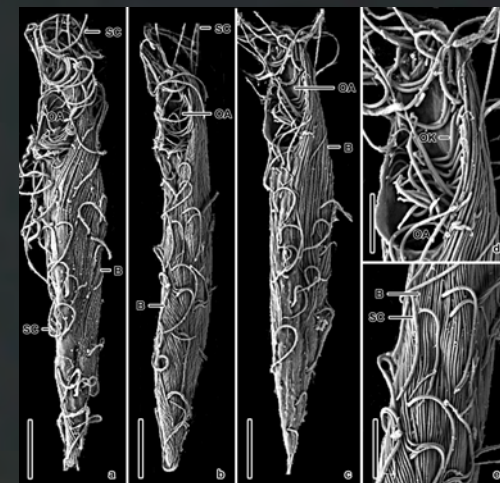
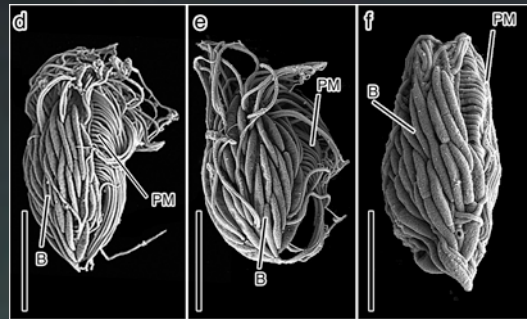


- CARD-FISH and 16S rRNA confirm at least two sulfate reducers (Desulfobulbaceae, Desulfobacteraceae), a Bacteroidete, methanogen (Methanobacteriales?)
- Internal membranes of one morphotype reminiscent of Type I methanotroph
- ~ 71/cm³ anoxic sediment

The permanently stratified Cariaco Basin, Venezuela - a natural laboratory for studying the microbiology of stratified water columns



Deep sea brine pools at bottom of Mediterranean Sea



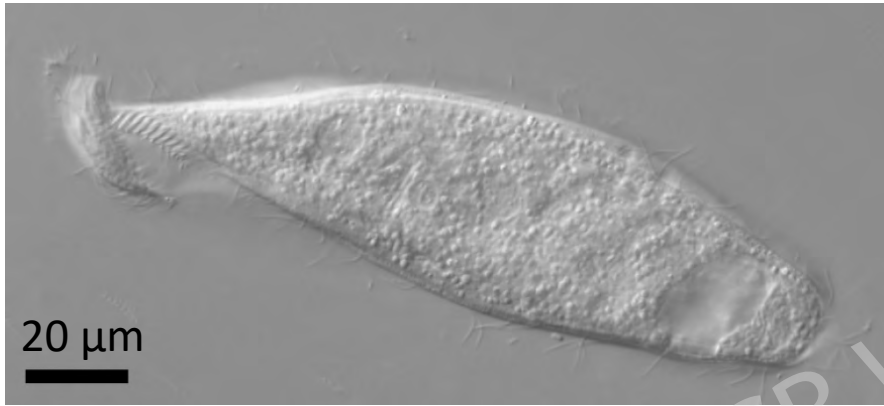
Symbiosis may be an adaptation to low oxygen/
sulfidic habitats

In addition to the free-living bacteria and archaea, the collective activities of 100-1000 symbionts/host cell likely contribute significantly to carbon and other nutrient cycling where hosts are abundant

How do these associations and their activities change along oxygen gradients?

A look at metabolic coupling between *Metopus* sp. strain CSS (Armophorea) and methanogen symbionts

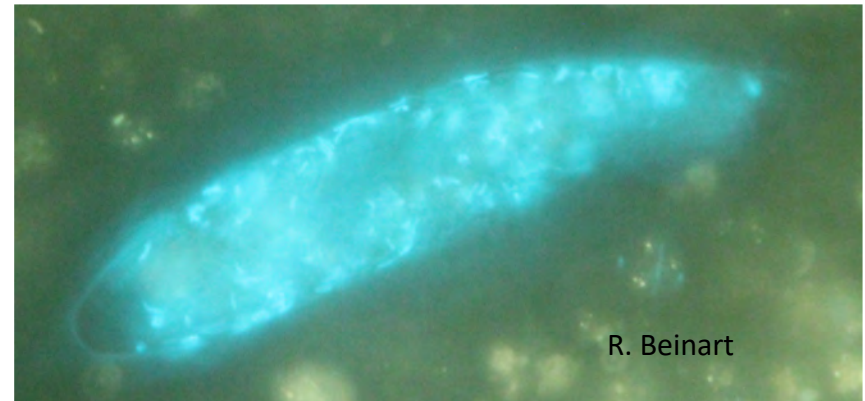
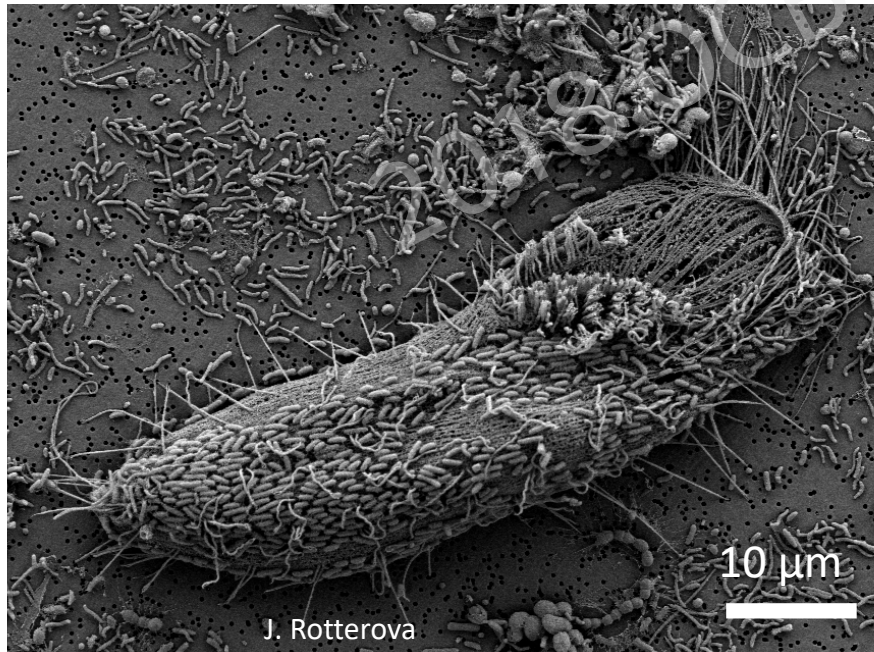
R. Beinart



Isolated by J. Rotterova & I. Cepicka,
Charles U.

Isolated from anoxic sediments in
Jetřichovice, Czech Republic.

Hosts dense populations of
intracellular methanogens (and some
surface deltas?)



Assessing the activity of this ciliate microbiome using experimental measurements of metabolism and comparative genomics

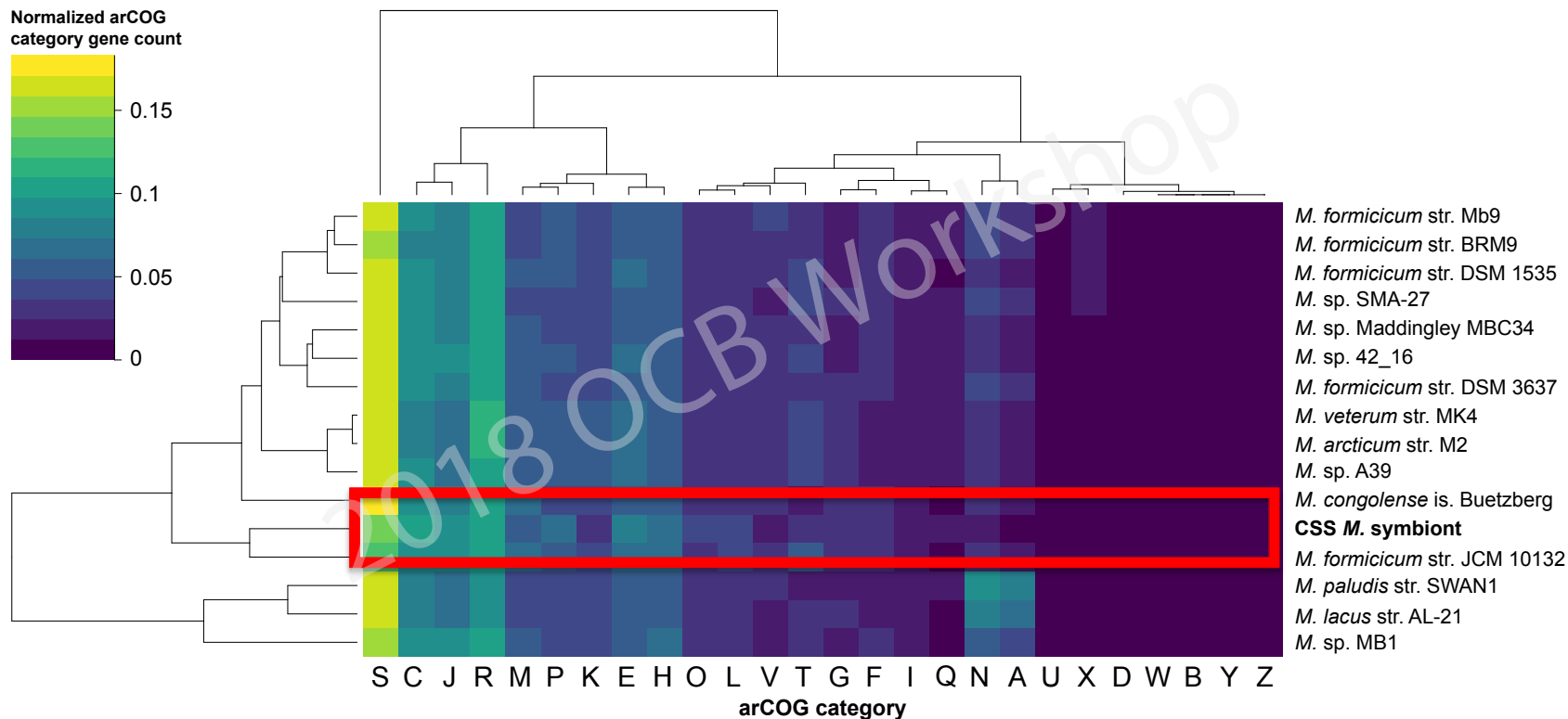


Metopus sp. (strain CSS)

Metagenomics of CSS

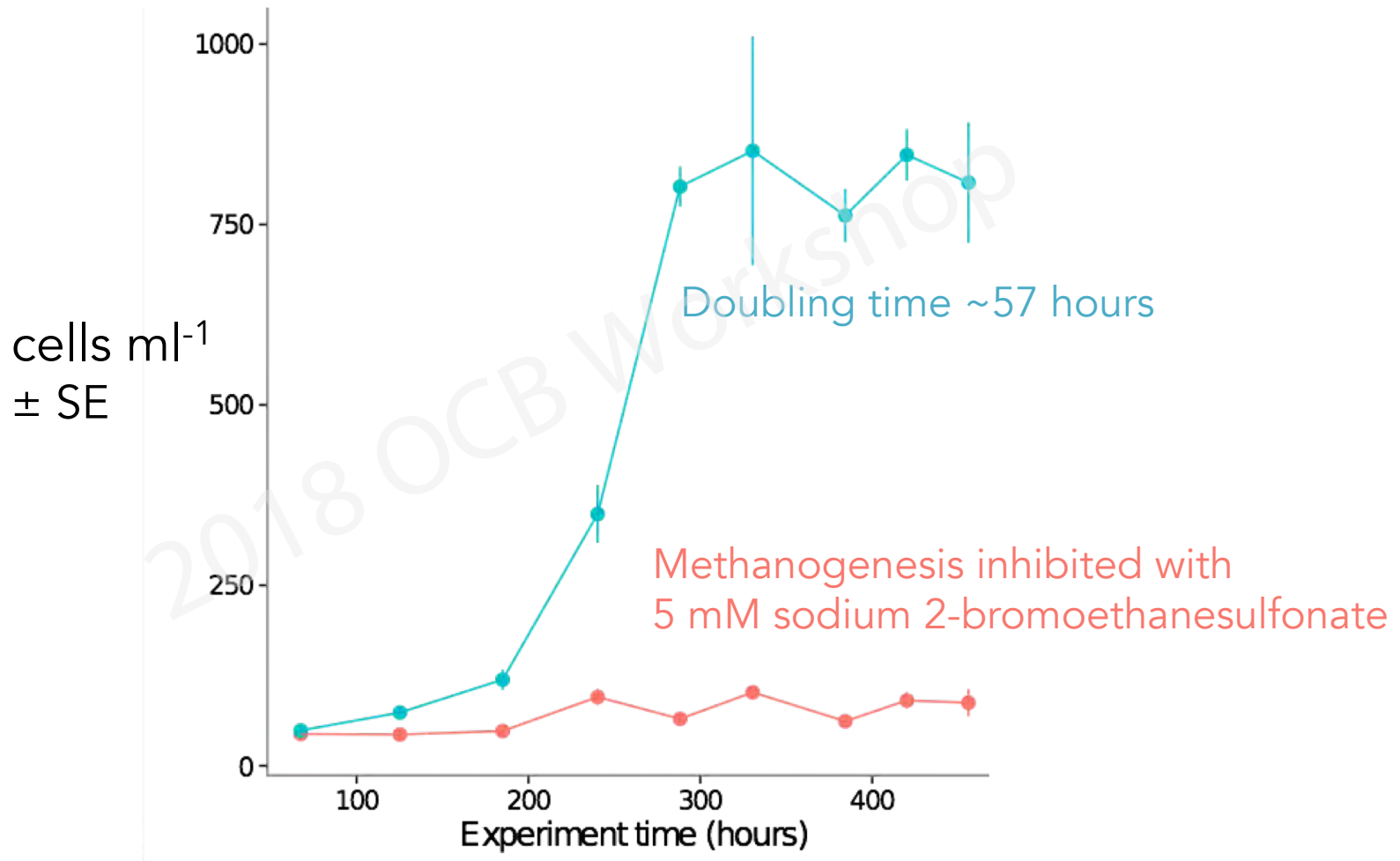
- ~ 60 cells hand picked, starved, and washed
- CSS and no-cells wash control sequenced using Illumina NextSeq
- Libraries for CSS and the wash controls co-assembled and binned
- Reads mapped to bins ID'd to *Methanobacterium*, a heterometopid ciliate, and a bin for "other"
- Reads mapping to *Methanobacterium* reassembled and bin is 2.02 Mb (N50=2976), 93.2% complete, 0.04% contamination
- Size not dramatically different from other M. genomes, but higher gene density

Hierarchical clustering of gene counts within each arCOG category normalized to # of arCOG annotated genes in each genome

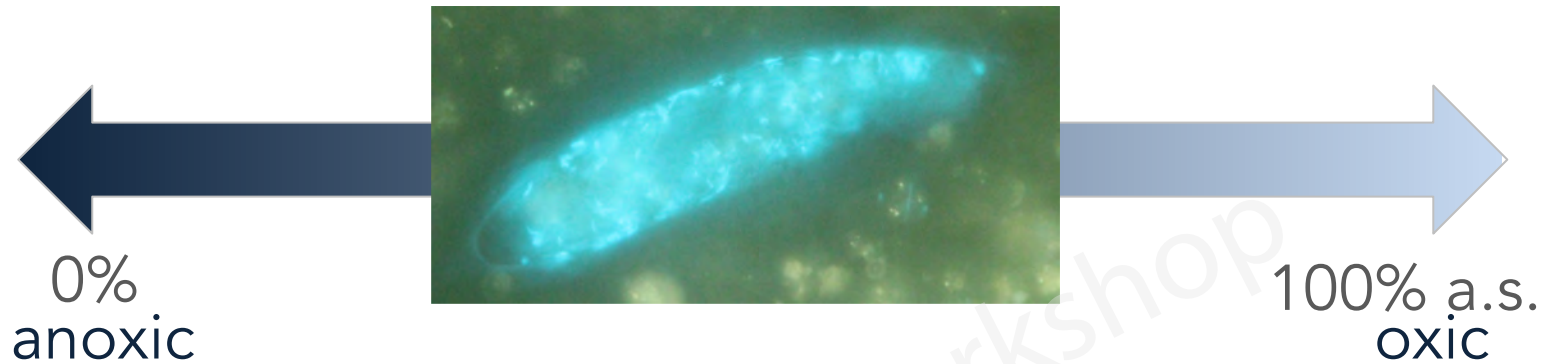


CSS symbiont genome has **more** genes involved in 'Inorganic ion transport and metabolism', 'Amino acid transport and metabolism', and 'Post-translational modification, protein turnover, and chaperones' and **fewer** genes in 'Transcription' and 'RNA processing and modification'.

Consistent with Fenchel and Finlay's work,
Methanogenesis is critical for this ciliate's growth



Host can tolerate a range of $[O_2]$ from anoxic to oxic



Headspace $[CH_4]$ via GC with TCD+FID
under varying dissolved O_2 conditions



Monitor O_2
with optode
spot



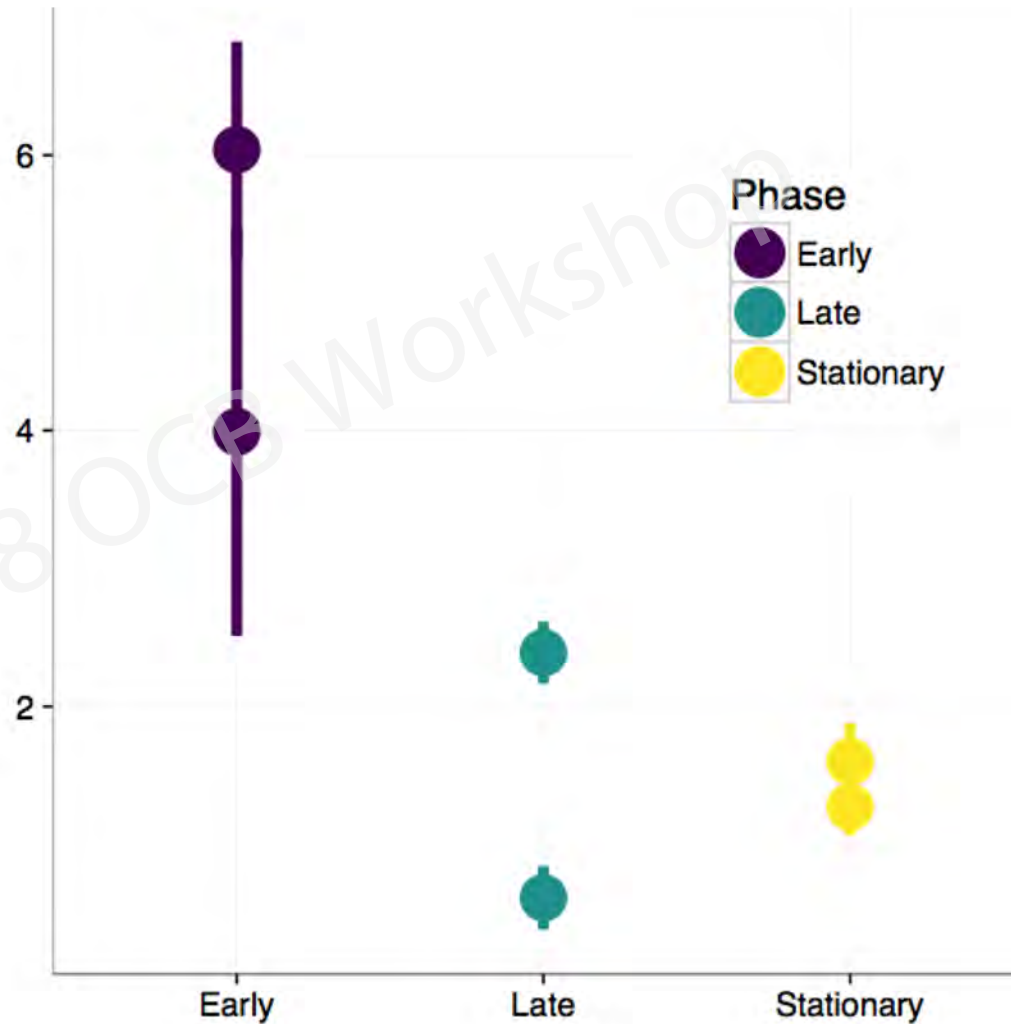
All experiments in triplicate

Because ciliate grown on mixed prey community:

1. Whole culture
2. No ciliates (3 μm filtrate)
3. Whole culture + BES, stops methanogenesis so we can observe methane loss over experiment

On a per host cell basis CH_4 production is dependent on host growth stage

Avg. rate of CH_4 production
(nmoles ciliate⁻¹ hr⁻¹)

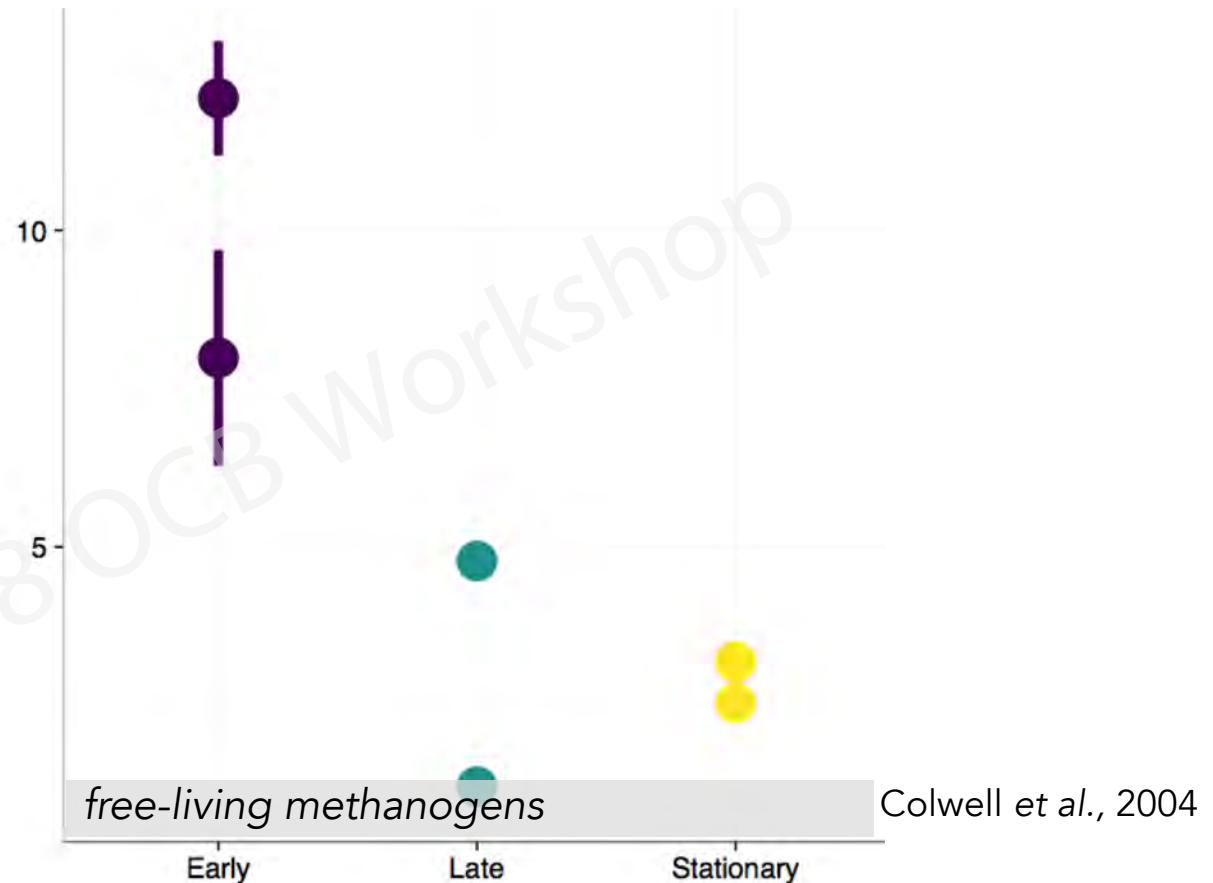


Estimated symbiont-specific CH₄ production is high relative to free-living methanogens

Avg. rate of
CH₄ production
± SE
(pmoles symbiont⁻¹ hr⁻¹)

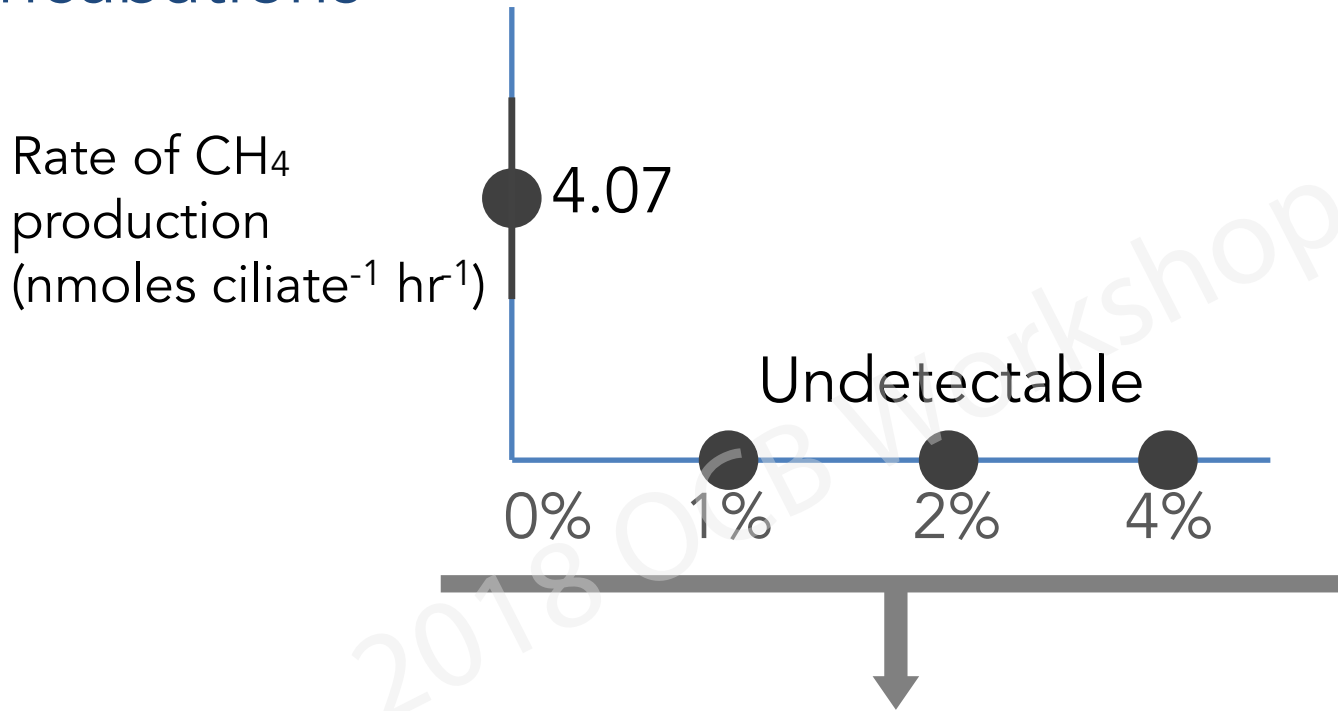
*Based on conservative
estimate of 500 cells ciliate⁻¹*

Actual numbers
~200 ciliate⁻¹



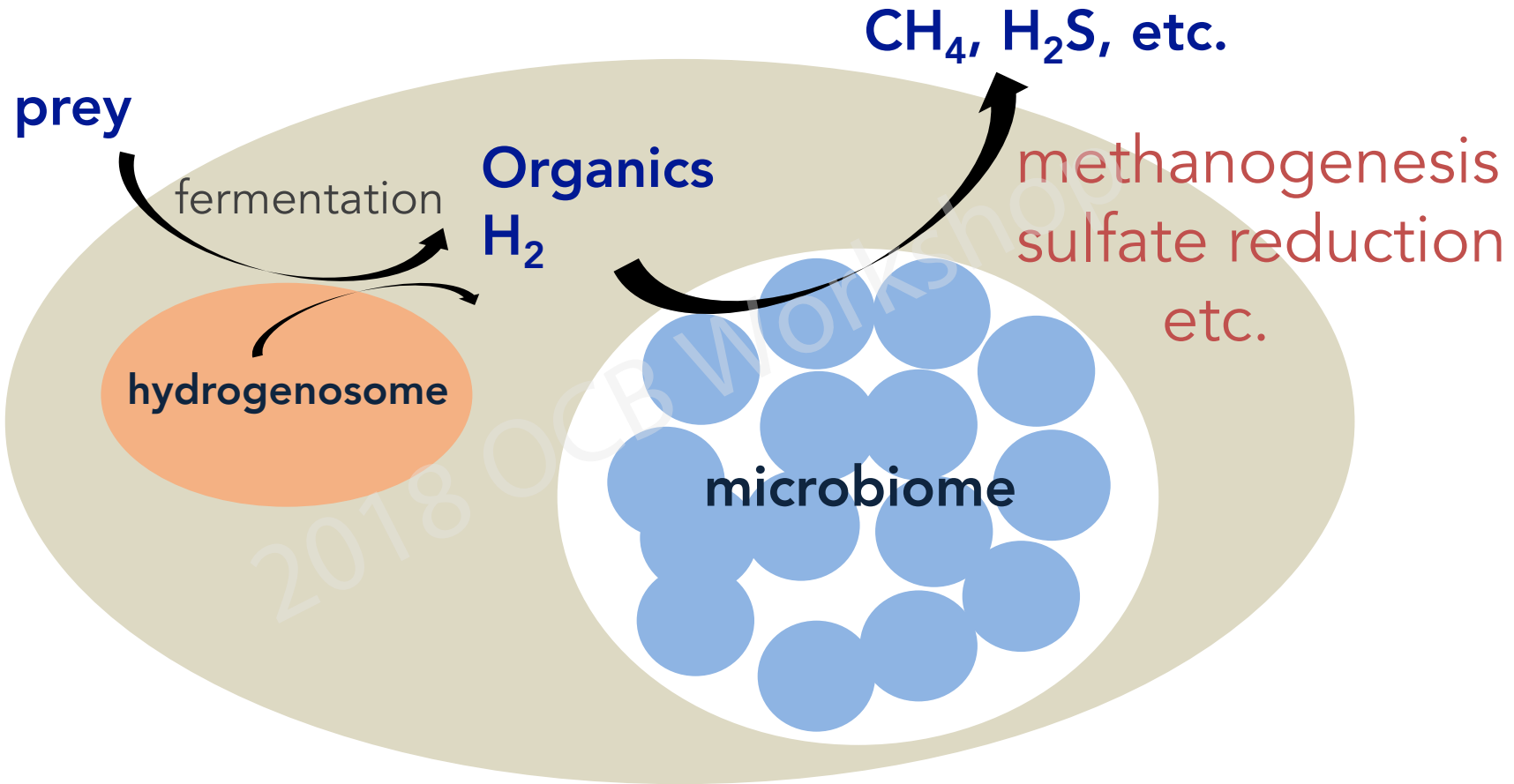
Transcriptome sequencing

[O₂] impacts methane production over 10-12 hour incubations



- BES-treated cultures headspace [CH₄] decreases with time under aerobic conditions – methane oxidizers?
- Methanogenesis may not occur above rates of [CH₄] oxidation in presence of O₂.
- Transcriptomes suggest expression of methanogenesis genes not significantly depressed under O₂ over 12 hours.

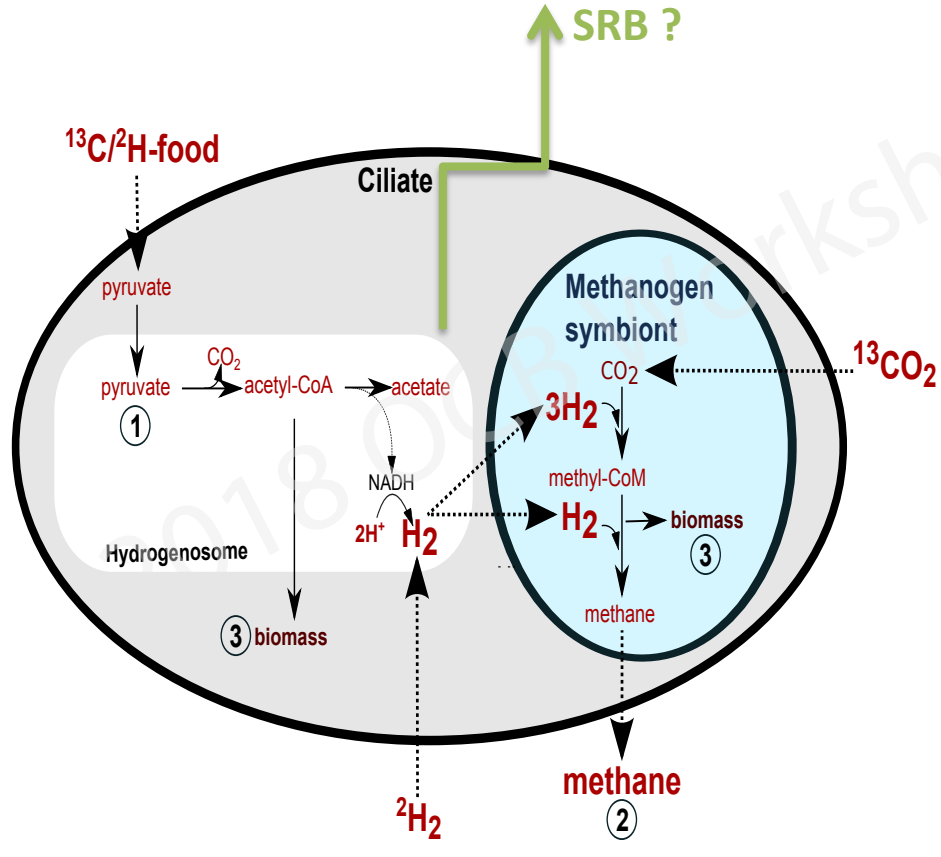
Proposed model for evolution of protist-microbiome interactions in anoxic habitats is based on H_2 transfer



Anaerobic protist

Untangling coupled metabolisms centered around H₂ transfer using new Raman methods

Collaborators: G. Taylor and Felix Webber (Stonybrook U.), J. Martinez (Bigelow Labs) with I. Cepicka and J. Rotterova (Charles U.), R. Beinart (URI), M. Pachiadaki (Bigelow Labs)



Simplified metabolic pathways of both host and hydrogen-utilizing methanogenic symbiont, entry points for stable-isotopically labeled food and substrates via Raman microspectrometry

Activity of protist microbiomes is likely significant and should be accounted for in estimates of key processes

- **Numbers of symbiont cells (100s-1000s per host cell)**
may rival or exceed free-living counterparts of same taxa
- **Host-provided substrates, niche, or positioning**
may increase rates
- **Co-evolution with host**
may drive selection for elevated metabolic rates

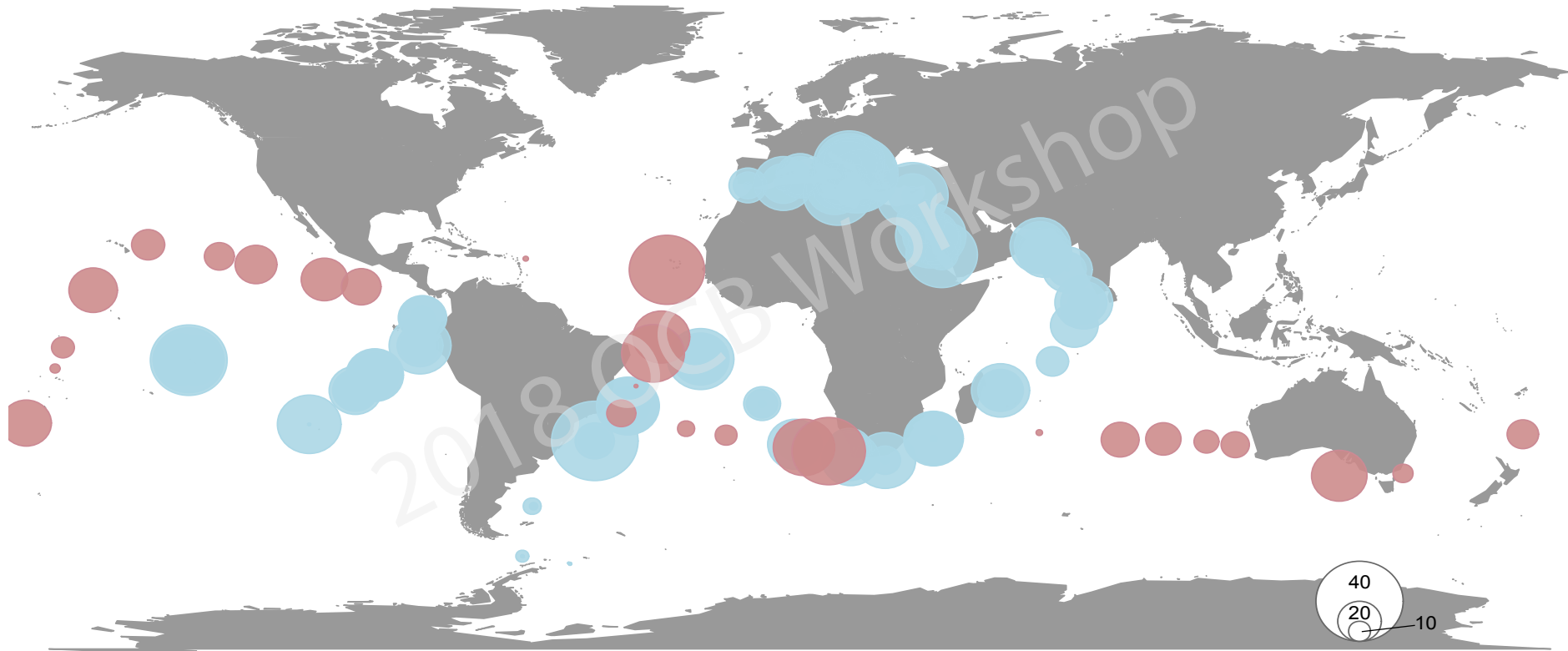


Parasitism and the Marine Carbon Cycle

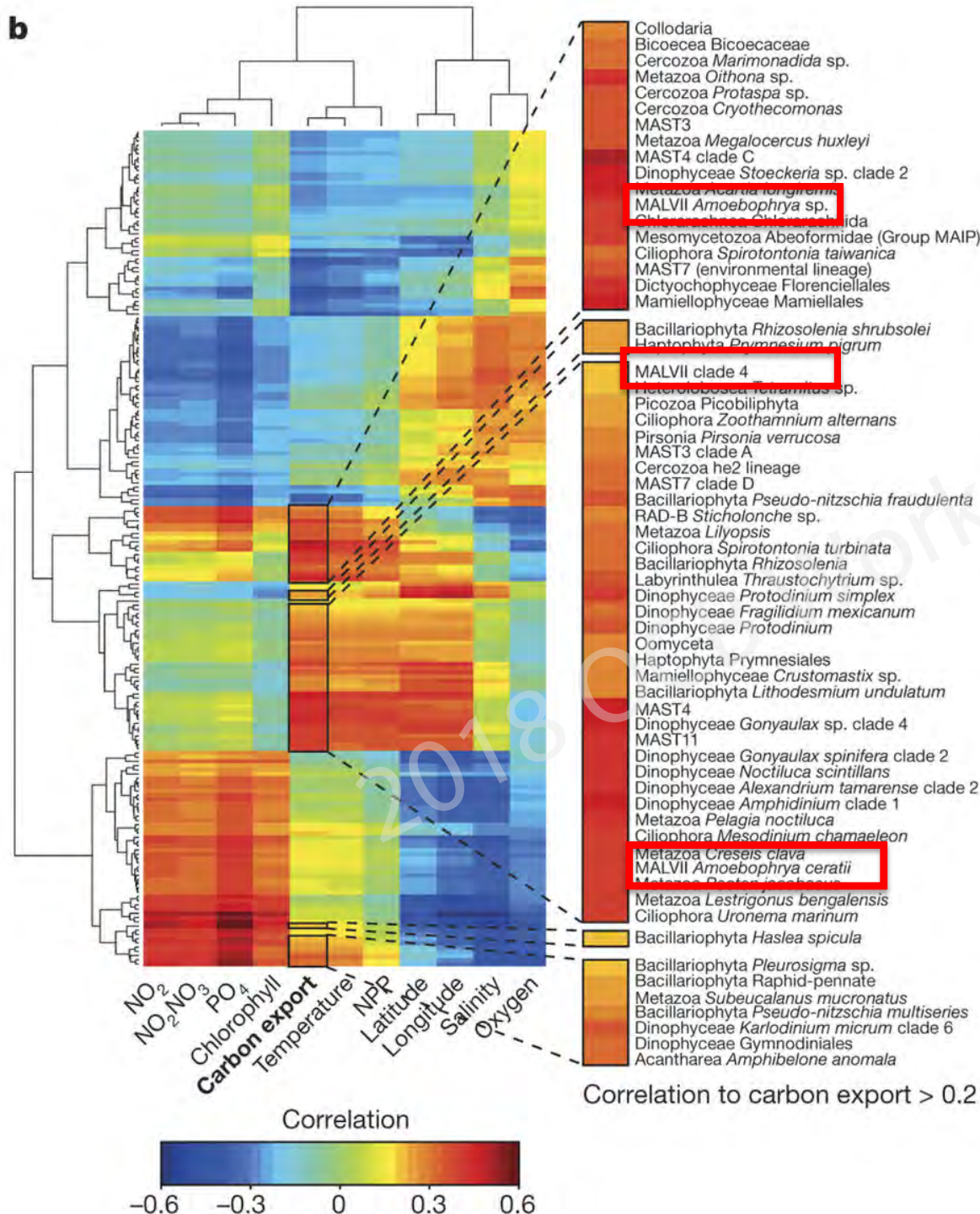
Protist parasites are abundant

Parasitism of plankton by eukaryotic parasites affiliated with the hyper-diverse Marine Alveolate Groups I and II (MALVI and MALVII) (Syndiniales) may make significant contributions to pools of particulate and dissolved organic carbon where hosts and parasites are abundant

Relative abundance (%) of all MALV (Syndiniales) subgroups in marker gene libraries from Malaspina (red, depths 2000-4000m) and Tara (blue, surface waters and DCM)



- Known hosts include diatoms, radiolarians, ciliates, copepods, and fish larvae.
- There is still a paucity of data regarding host-parasite specificity and infection dynamics.

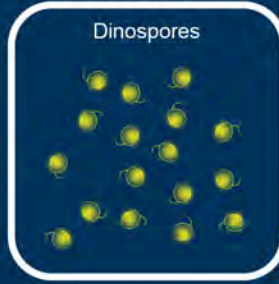


Clustering heatmap of regression-based modeling of Tara data highlight strong correlations of certain protist taxa and carbon export at 150m

Strong correlations between C export and **Alveolate parasites**

Infectious Stage

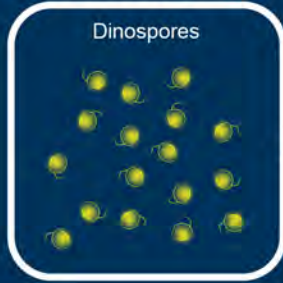
Dinospores



2018 OCB Workshop

Infectious Stage

Dinospores



Parasite Infection of Host

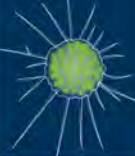


Hosts

Phototrophs



Mixotrophs



Heterotroph



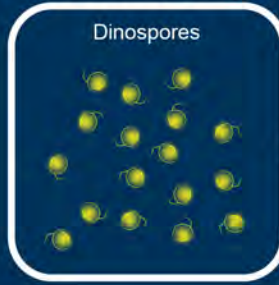
Zooplankton



2018 OCB Workshop

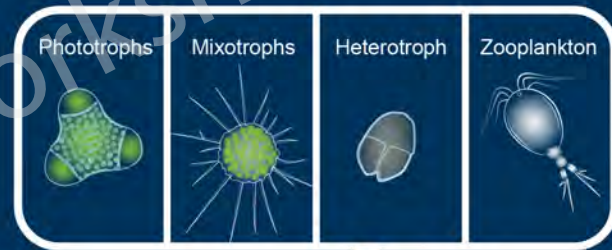
Infectious Stage

Dinospores



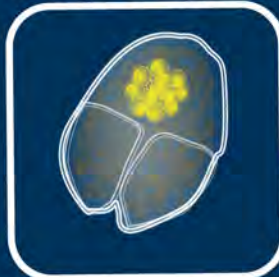
Parasite Infection of Host

Hosts

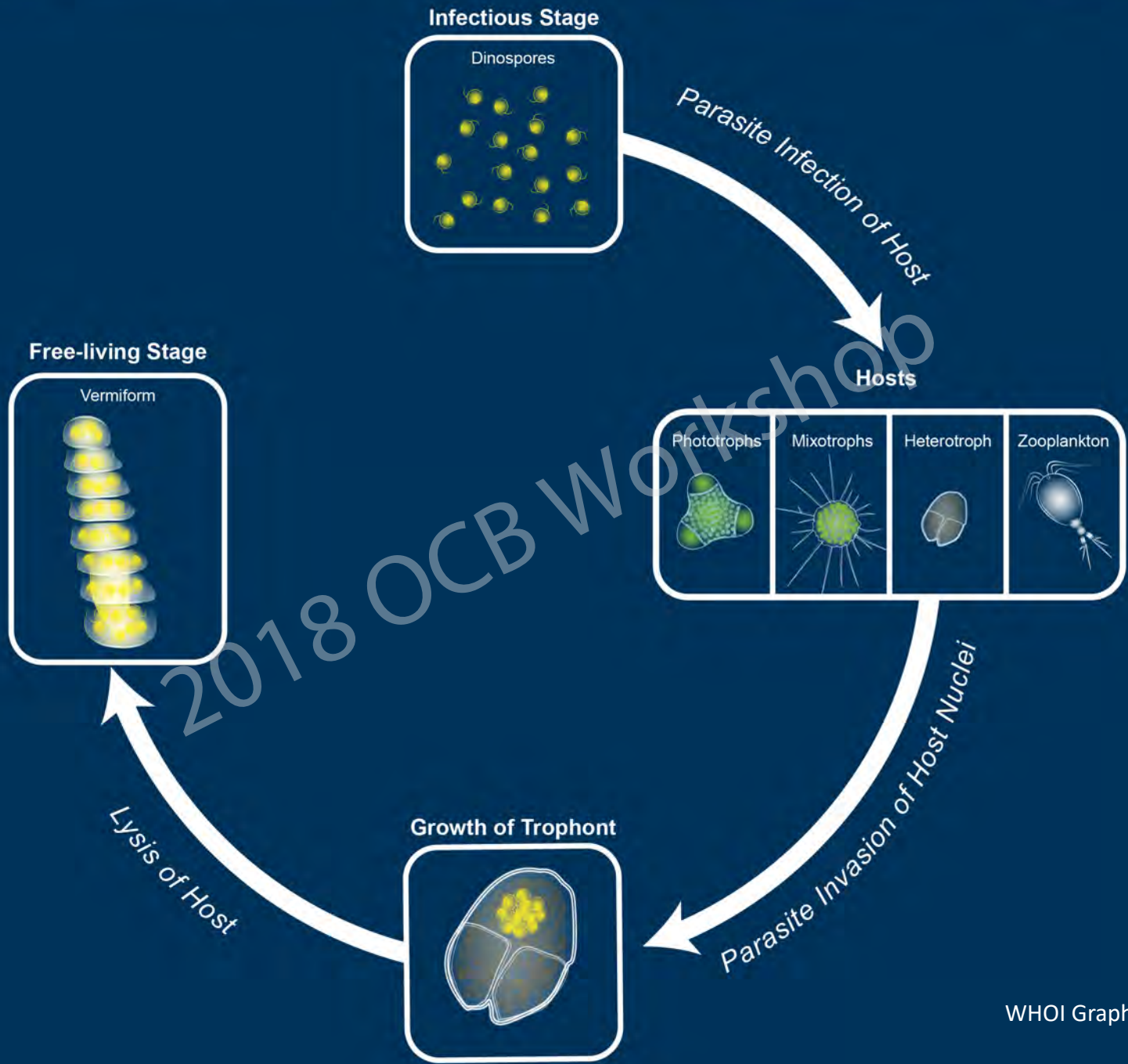


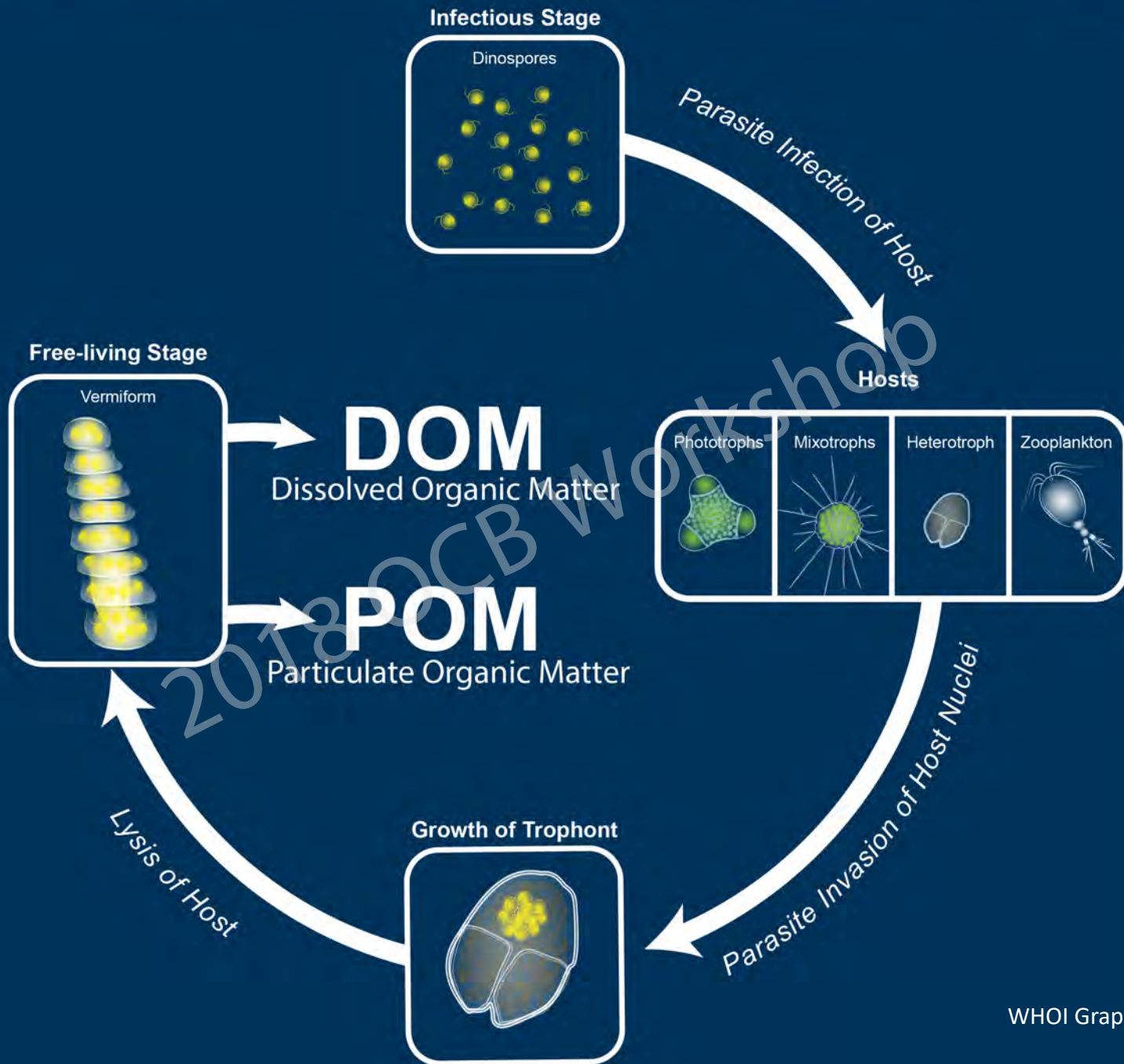
Parasite Invasion of Host Nuclei

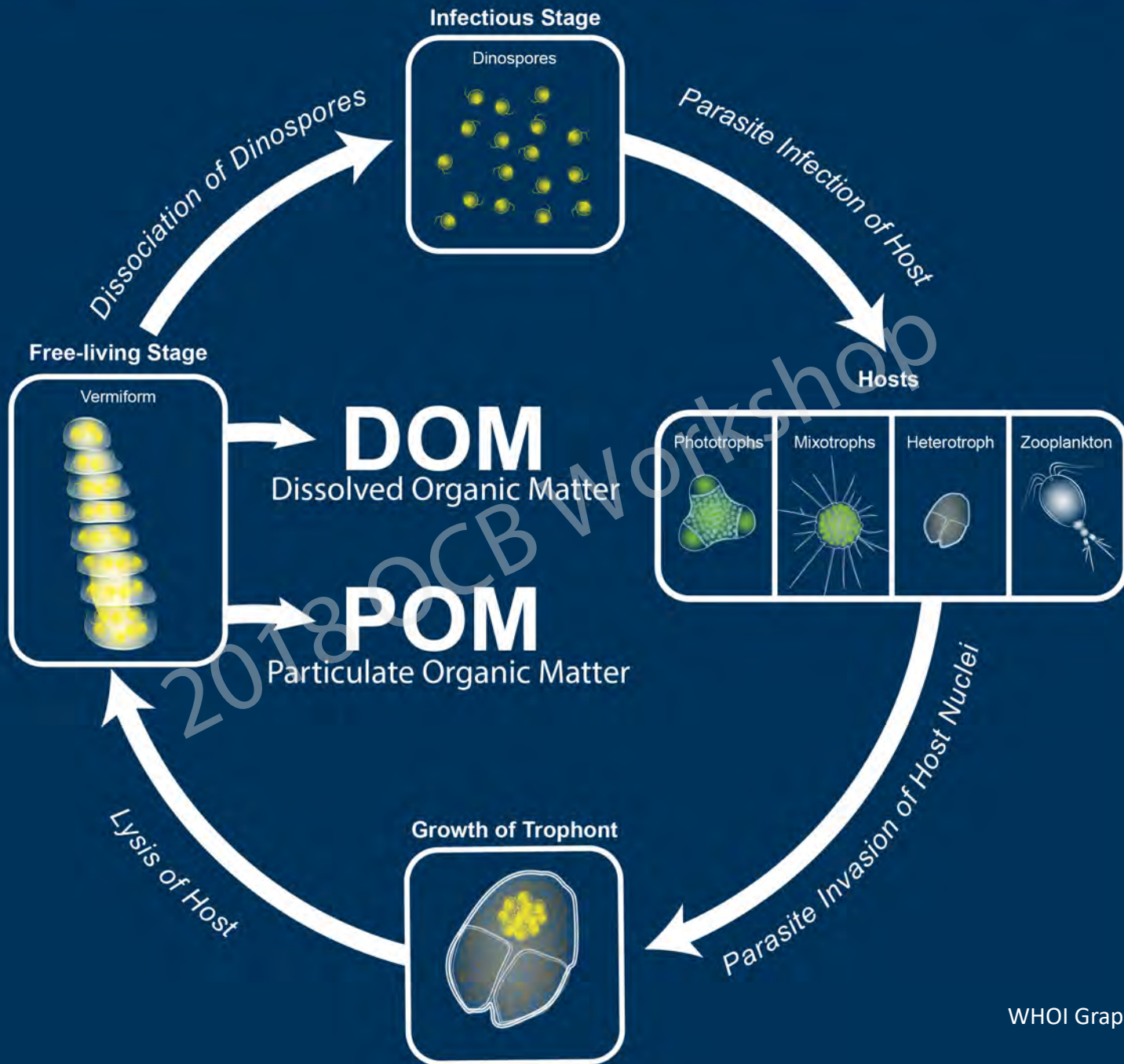
Growth of Trophont



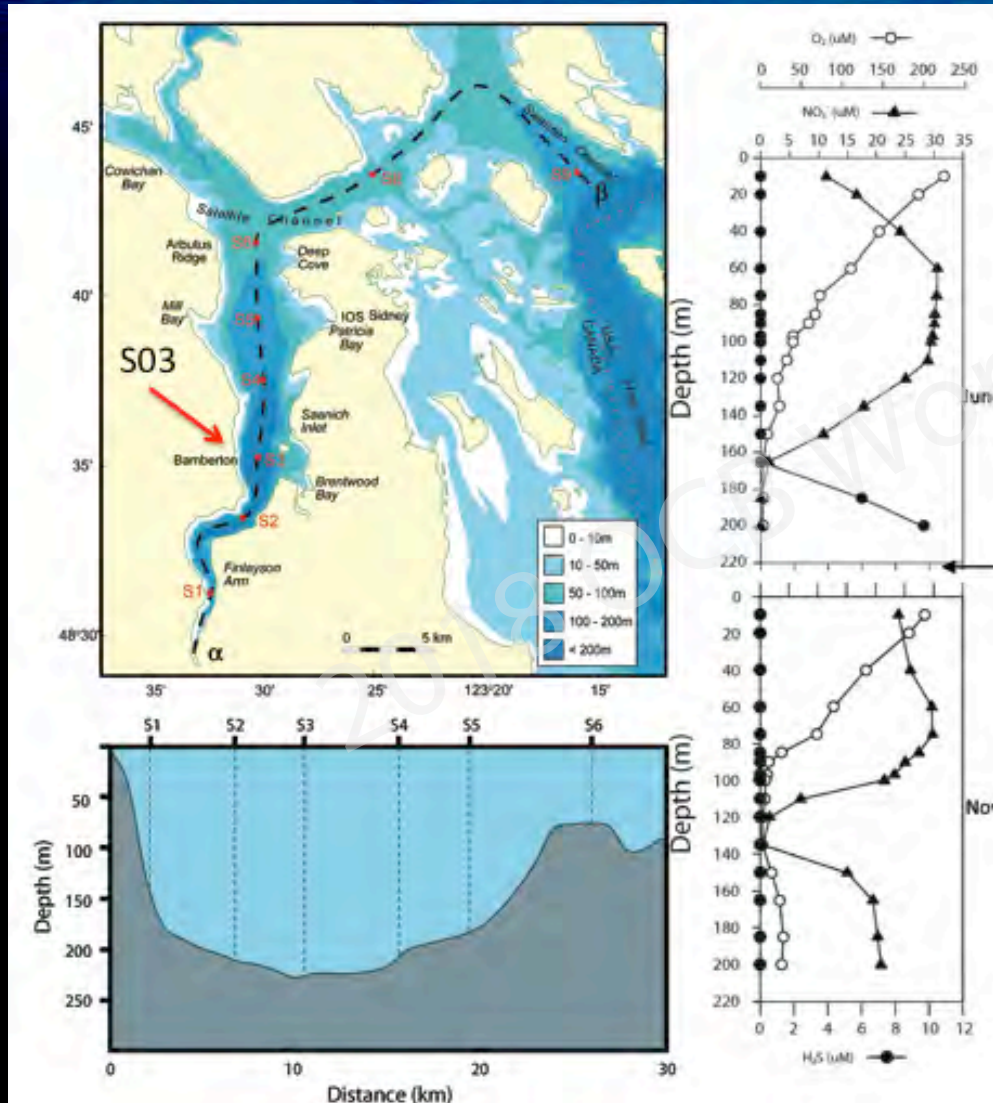
2018 OCB Workshop







Impact of Seasonal Oxygen Depletion in Saanich Inlet, Vancouver, BC: Collaboration with S. Hallam

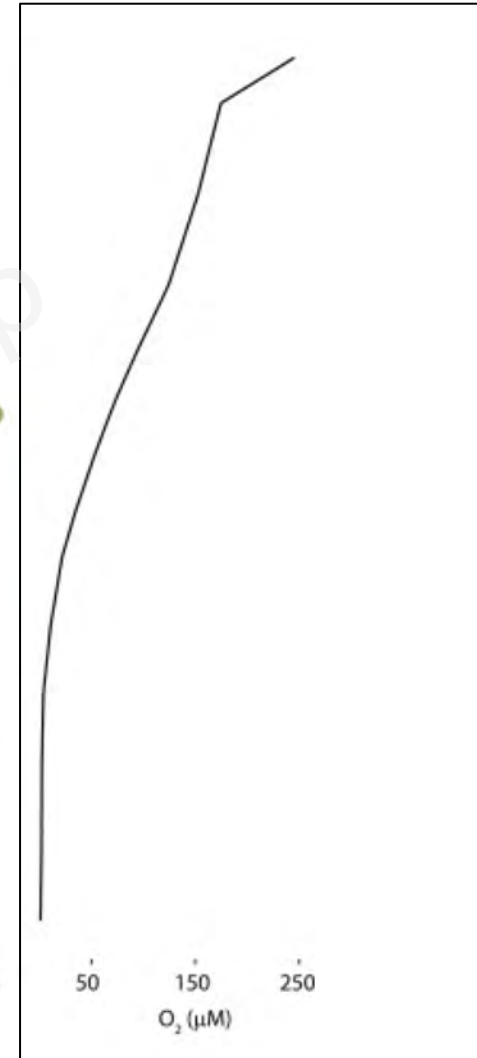
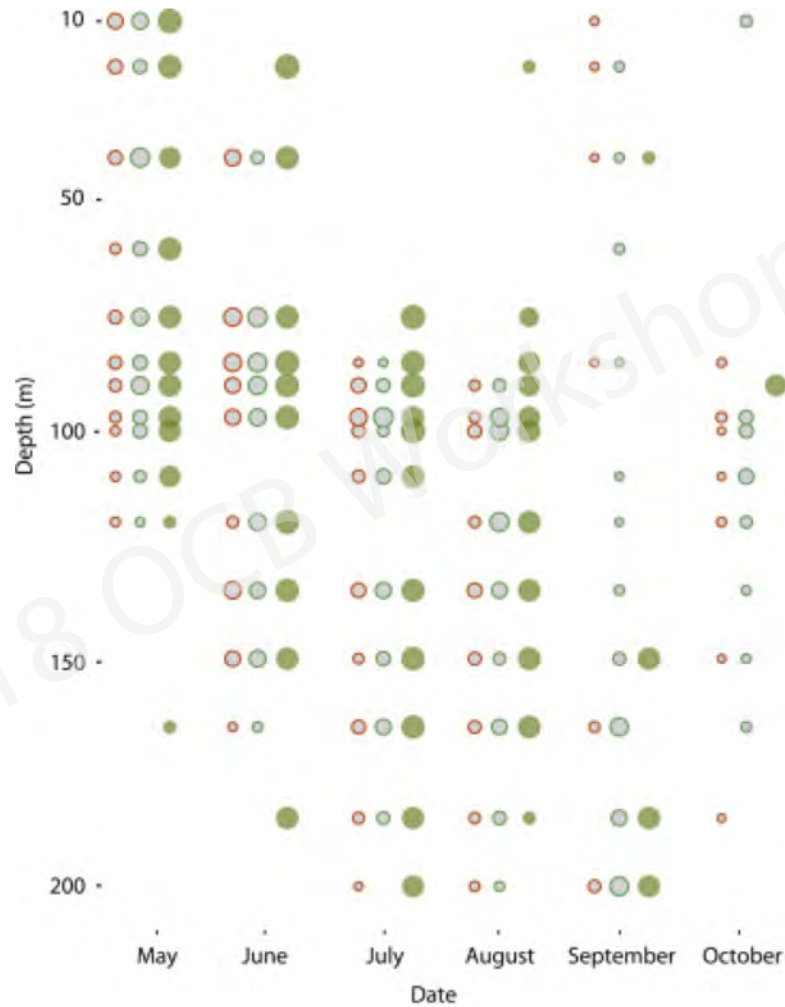


Multi-level indicator species analysis used to reveal OTUs occurring under specific O₂ conditions and protist co-occurrence patterns

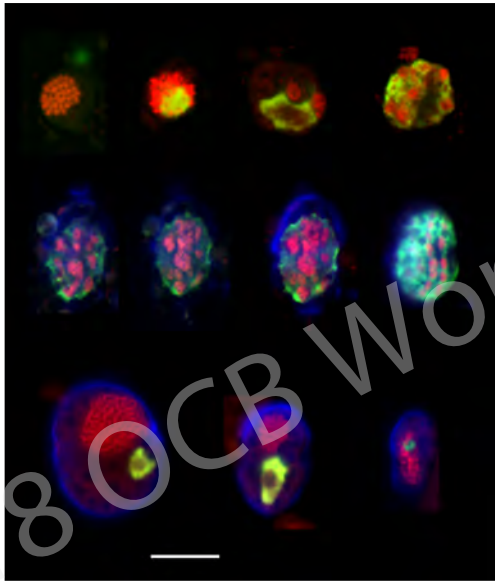
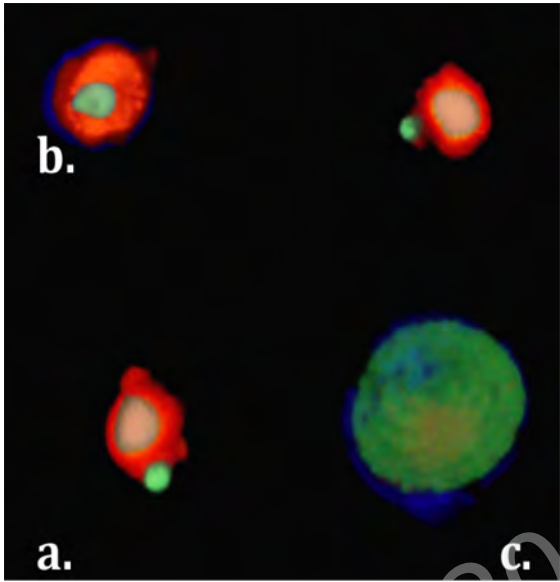
Syndiniales (MALVII) OTUs most abundant indicator OTUs during peak stratification

Phaeocystis antarctica Bloom Profile Mirrored by Syndiniales OTUs

- 18S iTag data capture a bloom of *Phaeocystis antarctica*
- Syndiniales OTUs follow *Phaeocystis* OTUs across depths and dates, suggesting infected cells could be sinking

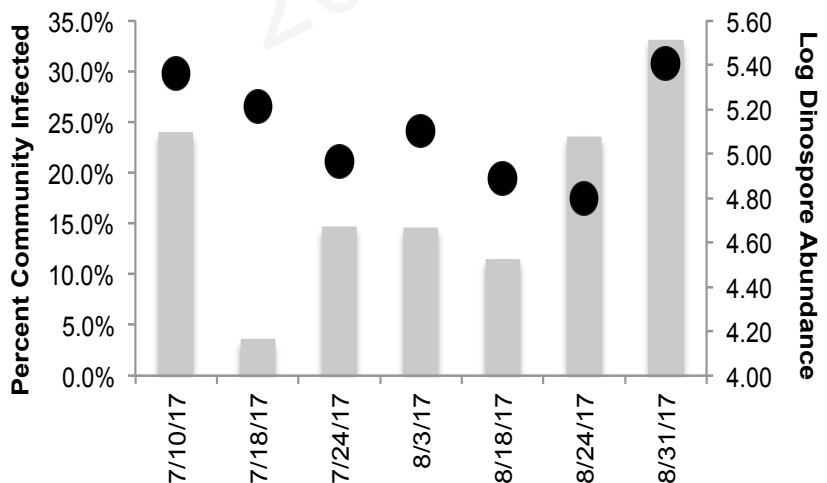


Investigating host-parasite dynamics and impacts on release of POC in a stratified coastal pond, Falmouth, Cape Cod, MA – Taylor Sehein (WHOI/MIT JP), L. Guillou (CNRS, Roscoff), R. Gast and M. Pachiadaki (WHOI)



Research Plan:

- Hi-res sampling
- Single-cell isolations/PCR amplifications
- Draft genomes
- Host and dinospore FISH
- Community marker gene profiling
- Lab studies of host specificity and carbon release



IN YOUR MEASUREMENTS/ ESTIMATES/
MODELS OF MICROBIAL PROCESSES
AND FLOW OF CARBON, DON'T FORGET
ABOUT THE PROTIST GRAZERS, THE
MIXOTROPHIC AND AUTOTROPHIC
PROTISTS, THE **PARASITIC PROTISTS**,
NOR THE **SYMBIONTS** OF PROTISTS THAT
MANY IGNORE OR FILTER OUT OF
SAMPLES!!!

Thank you for your attention!

Lab members past and present who contributed to this talk:

Roxanne Beinart (now at URI)

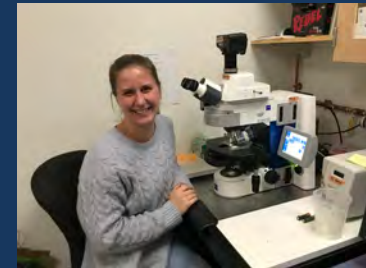
David Beaudoin

Taylor Sehein (WHOI/MIT Joint Program)

Maria Pachiadaki (now Bigelow)



R. Beinart



T. Sehein

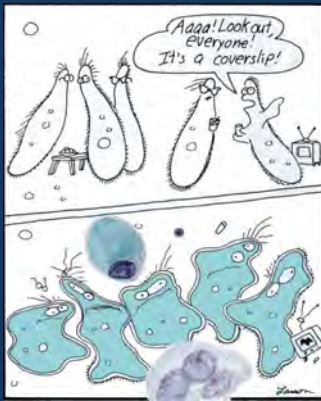
Ivan Cepicka, Johana Rotterova – Charles U., Czech Republic

Jeff Seewald (WHOI)

Steven Hallam, Monica Torres-Beltran (UBC, Canada)

Gordon Taylor, Elizabeth Suter, Felix Weber, T. Zaliznyak (Stony Brook U.)

Laure Guillou (CNRS, Roscoff, France)



GORDON AND BETTY
MOORE
FOUNDATION

